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Effect of adding sunflower seeds in broiler diet on serum lipids profile and some productive performance

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Abstract---The study determined the effect of the addition of 10,20 and 30 gm/kg of sunflower seeds (kernel) to diets for broiler chickens during 1–35 days of age on the blood serum chemistry. One hundred twenty one-day-old broiler chickens were assigned to eight groups (treatments) with two replicates (15 birds per cage). The experiment lasted 5weeks. Broiler chickens receiving diets supplemented with sunflower seeds (kernel). blood biochemical responses of broiler chicks to dietary supplementation with sunflower seeds (kernel) significantly increased HDL, phospholipids and decreased cholesterol, LDL of blood serum in broiler chicks. Adding sunflower seeds to broiler diet lead to improvement the productive parameter (body weight gain, live body weight, feed intake and feed conversion ratio).

Keywords---poultry, sunflower seeds, feed, protein, phospholipids.

Introduction

Chicken has a higher concentration of monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) than pork or red meat (PUFA). Alterations to poultry's lipid profile may have an effect on the meat's taste and nutritional value. (Mir *et al.*, 2017; Carmona *et al.*, 2019). Hematological parameters have been evaluated in many different species of animals, including poultry, by many researchers. These parameters are known to change with many factors, one of which is nutrition (Amakiri *et al.*, 2009). In chicken, lipids are mostly represented by triacylglycerol (TAG) and are firstly synthesized in hepatocytes and then stored in adipocytes. In comparison with mammals, there are some differences regarding lipid metabolism in chicken, such as the transportation of dietary lipids to the

liver, hepatic lipogenesis, and the presence of unique lipoproteins in the blood. It has been evaluated that the main proportion (70%) of fatty acid synthesis in chicken occurs in the hepatocytes through the process named lipogenesis, and only 5% happens in the chicken adipose tissue. However, the remaining (25%) of fatty acids come from the diet

Sunflower (*Helianthus annuus*) meal (SFM) has been found to be a plant source of protein that can be used in poultry feed in place of soybean meal (SBM). SFM is a better choice than SBM because it has more crude protein (280 – 420 g/kg), more methionine, and no anti-nutrients (Yusuf *et al.*, 2012). In SFM, the hulls can alter the fiber content, digestibility, and bioavailability of nutrients, and growth performance can be affected as a result (Karkelanov *et al.*, 2020). However, the solubility and concentration levels of fiber in poultry diets can have a significant impact on performance (Tufarelli *et al.*, 2018).

DHSS refers to the seed after de-hulling but before oil extraction when fiber is de-hulled, it is easier to digest because it has been stripped of most of its bulky components this means that when compared to SFM, DHSS has less fiber and more oil and protein (Nadeem, *et al.*, 2010; Zając, *et al.*, 2020). Addition of full-fat seeds into diets for broiler chickens can modify the content of nutrients in birds' muscles to a certain extent, yielding a product with desired nutritional and dietary properties (Kiczorowska *et al.*, 2015; Kiczorowska *et al.*, 2016; Apperson and Cherian 2017). The concentration of HDL cholesterol increased while the concentration of LDL cholesterol decreased with increasing content of SFM in diet (Rama Rao *et al.*, 2006). The oxidation of low-density lipoproteins (LDL) plays an important role in the development of atherosclerosis (Ulaiwi *et al.*, 2015). The final expression of growth is the product of interactions among genetic, nutritional, and environmental factors (Sahib *et al.*, 2021).

phospholipids (PL) has a multifunctional role in lipoprotein metabolism. In the circulation, phospholipids (PL) facilitates the transfer of phospholipids from the surface of TG-rich lipoproteins towards high-density lipoprotein (HDL) particles during intravascular lipolysis. Three actions of (PL) are indicated by bold arrows. First, (PL) is involved in hepatic VLDL assembly and/or secretion. Second, surface fragments formed during lipolysis of VLDL by lipoprotein lipase (LPL) are transferred by (PL) and contribute to pre- β -HDL (and α -HDL), together with nascent HDL secreted by the liver (and small intestine). Third, (PL) remodels mature plasma HDL by fusing typical α -HDL particles to form larger α -HDL, followed by shedding off pre- β -HDL (Van Tol, 2002).

The improved performance of broilers fed lower levels of SFM compared with the performance of control birds was related to cellulose content of the diet (Moghaddam *et al.*, 2012). Feed intake was determined as feed consumption from each treatment on daily basis by subtracting left over from feed served per group and adequate measures were taken to safeguard against spillage and related wastage. The mean daily feed intake was calculated on weekly basis by dividing the amount consumed by the number of birds in the group (Mohammed *et al.*, 2020).

Addition of full-fat seeds into diets for broiler chickens can modify the content of nutrients in birds' muscles to a certain extent, yielding a product with desired nutritional and dietary properties (Kiczorowska *et al.*, 2015; Kiczorowska *et al.*, 2016; Apperson and Cherian 2017). is largely due to dietary animal proteins, along with grain ingredients common in most poultry feeds (Perween *et al.*, 2016; Ross *et al.*, 2019).

Material and Methods

This experiment was carried out in the poultry farm of Veterinary Faculty, Baghdad University. The experiment was lasted for 35 days start from 06/04/2021 to 12/05 /2021. One hundred and twenty broilers (Rose 308) unsex chicks one day-old were raised in ground type housing and was divided randomly into 4 treatment groups (T1=Control,T2= 10gm,T3=20gm,T4=30gm sunflower seeds) of 30 birds, each control and treated group was further sub-divide into 2 replicates of 15birds per replicate. And then determine biochemical parameter.

On day 35, blood was collected from the wing veins into EDTA-containing vacutainers for the assessment of hematological parameters. The obtained serum measurement total triglyceride, cholesterol (total, low-density lipoprotein, and high-density lipoprotein) using spectrophotometric tests, phospholipids measurement by Phospholipid Elisa kit Serum preparation after collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuged again.

Determination of Serum Total Cholesterol

A colorimetric method to determine total cholesterol concentration in chickens serum, involves the use of cholesterol esterase (CE), the cholesteryl esters in serum are convert to cholesterol and fatty acids. Then the cholesterol is oxidized by cholesterol oxidase (CO) to Cholestenone and hydrogen peroxide. In the presence of the mixture of 4-aminoantipyrine (4-AA) and phenol are condensed by H₂O₂ (hydrogen peroxide) in the presence of peroxidase (POD) to produce the quinoneimine dye which indicates the concentration of cholesterol according to method by (Allain *et al.*, 1974).

Determination of Serum Triglycerides

Principle

Sample incubated with enzyme of lipoprotein lipase (LPL) and triglycerides transform to glycerol and free fatty acids according to method by (Klotzsch *et al.*, 1990).

Determination of Serum HDL-Cholesterol

The chylomicrons and lipoproteins of VLDL, and LDL were precipitated by adding of 4% phosphotungstic acid solution, which contains 10% magnesium chlorid

(pH 6.2). The supernatant obtained after centrifugation contains of the HDL concentration. The cholesterol can be determined by the complementary kit used in determination of total serum cholesterol as described by (Burstein *et al.*, 1970).

Calculation of Low Density Lipoprotein cholesterol

Low density Lipoprotein cholesterol (LDL-C) can be calculated mathematically from the total cholesterol, by using Friedwald's formula as described by (Friedewald *et al.*, 1972).

Calculation of Very Low Density Lipoprotein

Very low density lipoprotein (VLDL) concentration was calculated as one – fifth of the serum TG (Fiancis *et al.*, 2001).

$$\text{VLDL} = \frac{\text{TG}}{5} \quad \text{Normal value:}$$

VLDL- Cholesterol	> 55 mg/dl.
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Phospholipids

This test measurement by Phospholipid Elisa kit Serum preparation after collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuged again.

Procedure

1. Dilution of Standards Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube use two wells, total ten wells.
2. In the Micro Elisa strip plate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be 5 loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubation: incubate 30 min at 37°C after sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 µl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.

9. Coloring: Add 50 μ l Chromoge Solution A and 50 μ l Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 μ l stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Productive parameters

Live body weight

chicken were weighed every week in 1,7,14,21,28 and 35day average live body weights for each treatment were recorded.

Body weight gain

this were calculate calculated depending on the following equation: -

Weight Gain = body weight at the end of the period – body weight at the beginning of the previous period.

Feed intake

The given feed minus the retained feed

Feed conversion ratio =Average feed intake (g) / Average body weight gain (g) as recorded by (Al-Fayadh *et al.*, 1989).

Result and Discussions

Serum lipid profile

These results demonstrate there were highly significant ($P < 0.05$) differences in cholesterol concentration in 4th group (118.40 mg/dl) compared with 2nd, 3rd and control group (118.40, 98.20, 96.40, 106.00) Respectively. Chicks feed SFS showed significant ($P < 0.05$) increased in TG concentration in 3rd and 4th groups (143.20, 143.20) compared with 2nd and control (92.40, 122.40). the 2nd group (92.40) recorded the lowest significant ($P < 0.05$) decrease compared with all treatment.

Serum HDL showed there were significant ($P < 0.05$) increased in treatment 2nd, 3rd, 4th and control groups (80.88, 100.46, 94.22, 58.40) respectively. The table (4.7) showed serum LDL recorded significant ($P < 0.05$) decreased in 2nd, 3rd, 4th and control groups (21.20, 10.88, 22.60, 41.40) respectively. In the same table VLDL showed significant ($P < 0.05$) decrease in serum VLDL in 2nd (17.88) compared with 3rd, 4th and control groups (28.92, 27.48, 24.46) respectively. There were superiority of 3rd in HDL and LDL they recorded (100.46, 10.88) respectively

Table 1: The effect of different level of SFS on cholesterol (mg/dl),TG(mg/dl), HDL(mg/dl), LDL(mg/dl) and VLDL(mg/dl). (mean±SE)

Group s	Cholesterol mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
T1	106.00±1.87 b	122.40±2.46 b	58.40±3.84c	41.40±0.74 a	24.46±0.76b
T2	96.40±0.92c	92.40±5.92c	80.88±6.00b	21.20±0.86 b	17.88±1.24c
T3	98.20±0.86c	143.20±6.88 a	100.46±4.16 a	10.88±1.05 c	28.92±1.16a
T4	118.40±2.92 a	143.20±9.55 a	94.22±3.39a b	22.60±0.74 b	27.48±2.10a b
LSD	5.54	20.1	13.38	2.58	4.21

Means with a different letter in the same row are significantly different (P<0.05)

Total cholesterol concentrations are influenced by the composition of the dietary fat and fatty acid composition, broiler muscle total cholesterol, total fat, and fatty acid composition are all affected by the amount and type of dietary fat and fatty acids consumed (Sandra and Batetta, 2004). The current result agree with Laudadio *et al.*, (2014) they recorded that the cholesterol and LDL was decreased while HDL increased significantly by inclusion of low-fiber SFM in the laying hens diet compared with those fed soybean-control diet.

The results of study agree with Rama Rao *et al.* (2006) they reported that increased content of SFM (≥67% of SBM) in the broiler diets had increased the concentration of HDL-C, while the concentration of low-density lipoproteins cholesterol (LDL-C) decreased. they also noticed that the serum protein and triglycerides concentration decreased progressively with the increase in dietary SFM.

The result of this study disagree with Fiky *et al.*, (2020) they showed that adding linseed or sunflower oils have significantly (P ≤ 0.05) increased cholesterol value at 5 weeks of ages. The obtain result disagree with Attia *et al.*, (2016) they noted that the dietary intake of different levels of sunflower meal did not reveal any significant difference (P>0.05) in the measured serum lipid parameters (total lipid, total cholesterol, high, low and very low density lipoprotein cholesterols. Even if the diet had no effect on VLDL and HDL plasma levels, the finding of higher plasma total cholesterol and phospholipids levels in chickens fed the low lipid diet compared with chickens fed the high lipid diet suggests that the lipid composition of lipoproteins was affected by the dietary energy source as diets were isoenergetic and isoproteic and had no effect on the amount of feed ingested (Baéza *et al.*, 2015)

Phospholipids Levels

The effect of adding SFS on phospholipids concentration obtained significance (P<0.05) increased in 4th group compared with 2nd 3rd (1050.20, 941.20, 1000.40) respectively. There was superiority of 1st group it recorded lowest value (919.20).

Table 2 : Effect of adding of (SFS) level on phospholipids concentration (mg/l) in broiler chicks (mean±SE)

Groups	Phospholipids (mg/l)
T1	919.20±7.18c
T2	941.20±6.88bc
T3	1000.40±29.17ab
T4	1050.20±31.53a
LSD	66.10

Means with a different letter are significantly different (P<0.05)

Phospholipids, as an essential class of lipids, regulate tissue and organ building by proteins at membrane hormonal and membrane-enzyme levels, as well as are involved in the relationship of fatty acids in the blood system, and provide interconnection of neutral fats and cholesterol with the globulins and albumins, thus defining synthetic and structural functions of lipoproteins (Kolesnik and Derkho 2016). The result of study agree with Al-Sultan, S. I. (2005). Who noted that the effects of feeding of increasing level of fish oil with cotton seeds caused phospholipids levels were significantly (P< 0.05) increased. The result of this study disagree with (Soltan *et al.*, 2008) detect that anise seed supplementation had no significant effect on serum phospholipids of broiler chickens when compared with the control .

The obtain result disagree with (El-Bagir *et al.*, 2006) they discover that the feeding black cumin seeds caused significant reductions in the serum phospholipids. It is not known how black cumin seeds lower the contents of phospholipids in serum, but it is tempting to speculate that a component of the seeds inhibits in the liver the flux of acetyl-CoA into the lipogenic pathway.

Productive performance

Live Body Weight

The results in table (3) showed the treated groups recorded significant (P<0.05) increased in body weight compared with control during the whole period

Group s Weeks	T1=Control	T2=10 gm (SFS)	T3=20 gm (SFS)	T4=30 gm (SFS)
W1	C168.00±0.70e	AB193.60±4.13 e	A204.40±1.36e	B183.60±1.99e
W2	C425.60±1.60d	AB485.00±1.48 d	A492.00±1.78d	B462.80±2.15d
W3	D853.20±1.39c	B953.00±0.70c	A1000.00±2.12 c	C938.20±1.77c
W4	C1664.20±17.25 b	B1714.60±3.64 b	A1783.30±1.56 b	D1584.00±3.58 b
W5	C2033.40±9.21a	B2140.20±2.00	A2242.42±3.22	C2042.40±4.44

		a	a	a
LSD	12.11			

Table 3 The effect of sunflower seed(SFS) on live body weight in broiler chicks (mean±SE)

Means with a different small letter in the same column are significantly different (P<0.05) Means with a different capital letter in the same row are significantly different (P<0.05)

Body weight gain

Body weight gain recorded significant (P<0.05) increase in body weight gain during all weeks due to adding SFS to the diet in all treated group compared with control (833.00, 844.40, 641.08, 812.66) respectively.

Table 4 The effect of sunflower (SFS) seed on Body weight gain in broiler chicks (mean±SE)

Groups Weeks	T1=Control	T2=10 gm (SFS)	T3=20 gm (SFS)	T4=30 gm (SFS)
W1	D135.06±0.94e	B168.00±1.65e	C158.80±1.42e	A172.20±1.79e
W2	C307.06±3.66d	B355.20±3.75d	A361.20±2.45d	D295.06±2.45d
W3	C439.06±10.21c	A568.40±3.22c	B564.00±4.92c	D435.60±5.44c
W4	B615.06±4.08b	B614.00±4.82b	A702.00±3.12b	C462.01±2.67b
W5	C812.66±8.25a	B833.00±4.42a	A844.40±3.69a	D641.08±4.58a
LSD	2.15			

Means with a different small letter in the same column are significantly different (P<0.05) Means with a different capital letter in the same row are significantly different (P<0.05)

Feed Intake

feed intake in whole treated group with sunflower seeds (10mg, 20mg and 30mg) there were significant (p≤0.05) increased compared with control (642.00, 702.00, 641.08, 630.00) respectively.

Table 5 : The effect of sunflower (SFS) on feed intake in broiler chicks (mean±SE)

Groups Weeks	T1=Control	T2=10gm (SFS)	T3=20gm (SFS)	T4=30gm (SFS)
W1	D118.28±0.54e	A157.28±1.55e	B154.75±1.21e	C132.00±1.79e
W2	C265.06±0.66d	B285.05±2.33d	B283.22±1.45d	A295.06±2.45d
W3	C402.00±11.21c	B476.52±3.82c	A504.40±3.92c	B435.60±5.44c
W4	D428.12±1.08b	A502.80±4.22b	A519.20±2.32b	C462.01±2.67b
W5	C630.00±7.25a	B642.00±3.42a	A702.00±3.78a	B641.08±4.58a
LSD	1.95			

Means with a different small letter in the same column are significantly different ($P < 0.05$). Means with a different capital letter in the same row are significantly different ($P < 0.05$)

Feed Conversation Ratio

Feed Conversation Ratio (FCR) good indicator for economic because ability of chickens to convert of diet to live body weight. The result of feed conversation ratio show there were significant ($P < 0.05$) between T2,T3 significant improvement at the fourth and fifth week compared with T4 and control.(1.29, 1.20, 1.33, 1.28)

Table 6 The effect of sunflower (SFS) on feed conversations ratio in broiler chicks (mean \pm SE)

Groups Weeks	T1=Control	T2=10 gm (SFS)	T3=20 gm (SFS)	T4=30 gm (SFS)
W1	B1.13 \pm 0.02bc	C1.06 \pm 0.02c	C1.02 \pm 0.04e	A1.29 \pm 0.01bc
W2	B1.15 \pm 0.01b	A1.24 \pm 0.01ab	A1.27 \pm 0.02b	B1.18 \pm 0.05d
W3	B1.09 \pm 0.01cd	A1.19 \pm 0.02b	B1.11 \pm 0.02d	A1.25 \pm 0.02c
W4	B1.43 \pm 0.01a	D1.22 \pm 0.02ab	C1.35 \pm 0.01a	A1.51 \pm 0.02a
W5	B1.28 \pm 0.02d	AB1.29 \pm 0.01a	C1.20 \pm 0.02c	A1.33 \pm 0.02b
LSD	0.05			

Means with a different small letter in the same column are significantly different ($P < 0.05$)

Means with a different capital letter in the same row are significantly different ($P < 0.05$)

The use of full-fat seeds in broiler chicken meals can alter the nutrient content of the birds' muscles to some amount, resulting in a product with desired nutritional and dietary qualities. (Kiczorowska *et al.*, 2015; Apperson and Cherian 2017). Inclusion of SFM in broiler diets has been associated with increased digesta viscosity (Horvatovic *et al.*, 2015) due to the high NSP content in SFM (Sredanovi'c *et al.*, 2012). Increased digesta viscosity reduces the rate of digestion and absorption of nutrients, elevates microbial activity in the intestine, reduces FI, and increases moisture in the litter (Bedford, 2000). The hemicellulosic compounds are not digested by young birds, whereas adult birds are able to digest substantial amounts of hemicellulose (Selvaraj and Purushothaman 2004). Oilseeds also influence the digestive tract and digestion processes in poultry, this is connected with the relatively large amounts of dietary fiber in oilseeds, a significant part of which dissolves in water, forming highly viscous solutions.

This result agree with salari *et al.*, (2009) show that FFSS in the diet for 1 to 21 and 1 to 42 day increasing feed intake and weight gain. Many researcher indicate that using conventional SFM also show that high SFM inclusion levels depress growth performance of chickens (Waititu *et al.*, 2018) . The result of study disagree with Arija *et al.*, (1998) they noted that the Broilers fed with diets containing 50 g kg⁻¹ or more of FFSK had a slower rate of gain than those fed the control wheat-soybean diet. (Zajac *et al.*, 2020) noted the results of the research

showed that the tested full-fat seeds can be considered good dietary ingredients with a positive effect on the poultry production process and, consequently, on the dietary value of poultry meat. Peric *et al.* (2010), who found that the inclusion SFM in broiler diets decreased growth performance in terms of poor feed efficiency and growth rate.

Rama Rao *et al.* (2006) reported that increasing the inclusion level of SFM in broiler diets depressed digestibility of nutrients and energy, an effect that can be attributed to the high Non-starch polysaccharides (NSP) content in SFM (Malathi and Devegowda, 2001), which has anti-nutritional effects in poultry. These study adding sunflower (kernel) with low doses had low anti nutritional factor . Sunflower meal can replace up to 50% of soybean meal without depressing growth performance in either the starter or the finisher period. The authors suggest that the inclusion of sunflower meal in broiler diets does not affect productivity or barn hygiene management (Zajac *et al.*, 2020).

These factors can limit the potential of these seeds to be used in poultry production to a certain extent, such doubts were raised by Ryhänen *et al.* (2007) and Pekel *et al.* (2009). They reported affect of oilseeds in poultry diets on production effects, especially when used in high doses. They noted impaired feed conversion and decreased feed intake during the starter period. The better feed conversion ratio observed in the 15 and 20% SFS was due to the increase in the AME content of the broiler finisher diet. Although the diets were isocaloric, based on the AME content of SFS as determined in adult roosters, biological evaluation of the AME content of broiler diets indicated numerically higher AME content of 20% SFS groups. The higher AME in 20% group could be due to the associative effect of basal diet components and increased apparent fat availability with as level of fat increased up to 12% Although the feed conversion ratio was improved significantly, BW did not differ among the treatments. (Selvaraj and Purushothaman 2004). In other study Selvaraj and Purushothaman (2004) was indicate that SFS can be used up to 20% in broiler diets without affecting the production performance and the dressing percentage. Because the AME of SFS and fat and fiber digestibility's of diets with SFS increased with age.

Slightly different results were presented by (Amerah *et al.*, 2015) who supplemented broiler chicken diets with 5–6%, 8–10%, and 8–12% of sunflower meal. They observed no significant differences in the average body weight, feed consumption, feed efficiency mortality, and organ weight. Sunflower seeds also contain anti-nutritional components: linatin, cyanogenic glycosides, phytones, trypsin inhibitors, lignans, and saponins (Westbrook and Cherian 2019). Sunflower oil contains several phytochemicals capable of promoting food safety without causing any side effect on continuous use and the dietary inclusion of essential oil at basal diet in broilers is capable of maintaining good health Agubosi *et al.*, (2022).

Conclusion

This research demonstrates that including sunflower seeds (SFS) in the feed of broiler chickens has a beneficial impact on the animals' overall health, as measured by a drop in cholesterol and an increase in good cholesterol (HDL). A

beneficial effect is seen on phospholipids when 10 grams, 20 grams, or 30 grams of sunflower seeds (SFS) are included in the diet of broiler chickens.

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two and One-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. Also, independent t test was used to assess the difference between two means. $P < 0.05$ is considered statistically significant (SAS.2010).

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